

PCTWORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶: A61K 31/425	A1	(11) International Publication Number: WO 96/18395 (43) International Publication Date: 20 June 1996 (20.06.96)
(21) International Application Number: PCT/US95/15613 (22) International Filing Date: 12 December 1995 (12.12.95) (30) Priority Data: 08/357,121 15 December 1994 (15.12.94) US (60) Parent Application or Grant (63) Related by Continuation US 08/357,121 (CIP) Filed on 15 December 1994 (15.12.94) (71) Applicants (for all designated States except US): THE UPJOHN COMPANY [US/US]; 301 Henrietta Street, Kalamazoo, MI 49001 (US). BOEHRINGER INGELHEIM KG [DE/DE]; Postfach 200, D-55216 Ingelheim am Rhein (DE). (72) Inventors; and (75) Inventors/Applicants (for US only): HALL, Edward, Dallas [US/US]; 1432 Woodland Avenue, Portage, MI 49002 (US). VON VOIGTLANDER, Philip, F. [US/US]; 1 South Lake Doster, Plainwell, MI 49080 (US). ROHDE, Frank, A. [DE/DE]; Am Geistert 4, D-55413 Weiler (DE).	(74) Agent: WELCH, Lawrence, T.; The Upjohn Company, Corporate Intellectual Property Law, 301 Henrietta Street, Kalamazoo, MI 49001 (US). (81) Designated States: AL, AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TT, UA, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, LS, MW, SD, SZ, UG). Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>	
(54) Title: USE OF PRAMIPEXOLE AS A NEUROPROTECTIVE AGENT		
(57) Abstract The present invention provides the use of pramipexole as a neuroprotective agent.		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgyzstan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LU	Luxembourg	TD	Chad
CS	Czechoslovakia	LV	Latvia	TG	Togo
CZ	Czech Republic	MC	Monaco	TJ	Tajikistan
DE	Germany	MD	Republic of Moldova	TT	Trinidad and Tobago
DK	Denmark	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	US	United States of America
FI	Finland	MN	Mongolia	UZ	Uzbekistan
FR	France			VN	Viet Nam
GA	Gabon				

USE OF PRAMIPEXOLE AS A NEUROPROTECTIVE AGENT

FIELD OF THE INVENTION

The present invention relates to the use of pramipexole or 2-amino-6-n-propylamino-4,5,6,7-tetrahydrobenzo-thiazole, or the (+)- or (-)-enantiomers thereof,
5 and the pharmacologically acceptable salts thereof, as a neuroprotective agent.

BACKGROUND OF THE INVENTION

A number of central nervous system diseases and conditions result in neuronal damage. These conditions which can lead to nerve damage include:

Primary neurodegenerative disease; Huntington's Chorea; Stroke and other
10 hypoxic or ischemic processes; neurotrauma; metabolically induced neurological damage; sequelae from cerebral seizures; hemorrhagic stroke; secondary neurodegenerative disease (metabolic or toxic); Parkinson's disease; Alzheimer's disease, Senile Dementia of Alzheimer's Type (SDAT); age associated cognitive dysfunctions; or vascular dementia, multi-infarct dementia, Lewy body dementia, or
15 neurodegenerative dementia.

Pramipexole is a dopamine-D₃/D₂ agonist the synthesis of which is described in European Patent 186 087 and its counterpart, U.S. Patent 4,886,812. It is known primarily for the treatment of schizophrenia and Parkinson's disease. It is known from German patent application DE 38 43 227 that pramipexole lowers the plasma
20 level of prolactin. Also, this European patent application discloses the use of pramipexole in the treatment of drug dependency. Further, it is known from German patent application DE 39 33 738 that pramipexole can be used to decrease abnormal high levels of thyroid stimulating hormone (TSH). U.S. patent 5,112,842 discloses the transdermal administration of the compounds and transdermal systems
25 containing these active compounds. WO patent application PCT/EP 93/03389 describes the use of pramipexole as an antidepressant agent.

Up to now there is no commercially available drug for the therapeutic treatment of stroke with proven evidence of efficacy.

Surprisingly and unexpectedly, it has been found that pramipexole and its
30 (+)-enantiomer also has a neuroprotective effect.

INFORMATION DISCLOSURE

Piribedil, a vasodilator which binds to a multitude of receptors including dopamine receptors, is reported to have an effect on functional and biochemical parameters in a gerbil model of global cerebral ischemia. See, e.g., Society for
35 Neuroscience Abstracts, 19:673 (1993); *id.*, at 1645.

Lisuride binds to several different receptors including dopamine D₂ and 5-HT_{1a} receptors. It is reported that Lisuride, when administered before the event, reduced brain edema and prolonged survival time in a rat model of cerebral infarction. Miya Zawa, et al. Nippon-Yakurigaku-Zasshi 98(6):449-561, (1991).

5

SUMMARY OF THE INVENTION

The present invention particularly provides a method for preventing neuronal damage in a patient suffering from or susceptible to such neuronal damage comprising the administration of an effective amount of 2-amino-6-n-propylamino-4,5,6,7-tetrahydrobenzothiazole, its (-)-enantiomer or (+)-enantiomer thereof, and
10 pharmacologically acceptable salts thereof especially an effective amount of pramipexole which is the (-)-enantiomer of 2-amino-6-n-propylamino-4,5,6,7-tetrahydrobenzothiazole-dihydrochloride or an effective amount of the (+)-enantiomer of 2-amino-6-n-propylamino-4,5,6,7-tetrahydrobenzothiazole dihydrochloride.

15

Conditions which can cause nerve damage are well-known to an ordinarily skilled neurologist or similar physician and include:

Primary neurogenerative disease;

Huntington's Chorea;

Stroke and other hypoxic or ischemic processes;

20

Neurotrauma;

Metabolically induced neurological damage;

Sequelae from cerebral seizures;

Hemorrhagic stroke;

Secondary neurodegenerative disease (metabolic or toxic);

25

Parkinson's disease;

Alzheimer's disease, other memory disorders; or

Vascular dementia, multi-infarct dementia, Lewy body dementia, or neurogenerative dementia.

The preferred indication for pramipexole, in the context of the present
30 invention, is Parkinson's disease which is characterized by progressive degeneration of nigrostriatal dopamine neurons. In this sense, the term Parkinson's disease also comprises the term Parkinson's syndrome. In addition to pramipexole's palliative action (i.e. replacement of the lost dopamine neurotransmitter function), the compound may slow the degeneration of surviving dopamine neurons and thereby
35 slows the progression of the disease.

The prophylactic use of the compound of this invention includes use as monotherapy in early or pre-symptomatic stages of Parkinson's disease and prevention of neurodegenerative disorders based on ischaemia.

The synthesis, formulation and administration of pramipexole is described in
5 U.S. patents 4,843,086; 4,886,812; and 5,112,842; which are incorporated by reference herein.

2-Amino-6-n-propyl-amino-4,5,6,7-tetrahydrobenzothiazole, particularly the (-)-enantiomer thereof, and the pharmacologically acceptable acid addition salts thereof can be given for preventing of neuronal damage. The form of conventional
10 galenic preparations consist essentially of an inert pharmaceutical carrier and an effective dose of the active substance; e.g., plain or coated tablets, capsules, lozenges, powders, solutions, suspensions, emulsions, syrups, suppositories, etc.

L-dopa is shown to be toxic to cerebellar granule cells in culture. Pramipexole and the (+) enantiomer blocked L-dopa toxicity. The EC₅₀ for both was
15 between 0.3 and 1 uM and 10 uM provided viability measurements that were equal to control cells not exposed to L-dopa. The mechanism of protection does not appear to involve receptor activation given that the (+) enantiomer is less active in monoamine receptor binding assays. The possibility exists that 2-amino-6-n-propylamino-4,5,6,7-tetrahydrobenzothiazole, its (+) and (-) enantiomers and the
20 pharmacologically acceptable acid addition salts thereof, especially pramipexole and the (+) enantiomer are acting as antioxidants toward reactive oxygen species known to be generated from L-dopa incubation.

The effective dose range is 0.01 to 2.0 mg/kg. More preferred is a dose of 1-2 mg/kg PO. The preferred total dose level for neuroprotection is 0.5-20 mg/kg/day
25 PO. The preferred human dose is 0.1-6.0 mg/day total dose, divided in 2 or 3 administrations. In addition to being administered by oral or intravenous route, pramipexole may be administered transdermally.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention is seen more fully by the examples given below:

30 Example 1:

We examined histologically retrograde degeneration of the dopaminergic nigrostriatal (NS) tract over 28 days in gerbils subjected to a 10-minute period of forebrain ischemia via bilateral carotid occlusion. There was in control animals a 39% loss of NS cell bodies in the zona compacta (as judged by tyroxine hydroxylase
35 immunocytochemistry) over that time period. Daily post-ischemic oral dosing (1

mg/kg PO BID beginning at 1 hr after insult) with pramipexole attenuated 28-day post-ischemic loss of NS DA neurons by 36% ($p < 0.01$ vs vehicle-treated).

Example 2:

We have successfully replicated the finding of Example 1; i.e., that
5 pramipexole can protect nigrostriatal dopamine neurons in a gerbil model of 10
minutes of bilateral carotid occlusion-induced forebrain ischemia plus 28 days of
post-ischemic survival. The administration of pramipexole was via an initial dose of
1 mg/kg PO 60 minutes after ischemia and again at the end of day 1 followed by
twice daily dosing for the next 26 days. A twice daily dose of 0.3 mg/kg produced a
10 threshold effect (14% increase in nigrostriatal dopamine neurons compared to
vehicle). A twice daily dose of 1 mg/kg produced a significant 38% improvement in
28 day post-ischemic dopamine neuronal survival ($p < 0.0001$ vs vehicle-treated).
This action appears to be specific for dopamine neurons since the post-ischemic loss
of the non-dopaminergic neurons in the CA1 area of the hippocampus was not
15 significantly affected.

Example 3:

Primary Cultures of Cerebellar Granular Cells

Primary cultures of cerebellar granular cells were prepared from 8-day-old
Sprague Dawley rats (Charles River, Portage, MI) as described previously (1).
20 Neurons were grown on 6-well, 35 mm culture dishes (Nunc, Denmark) for 8-9 days,
2 ml/well, at a density of 1×10^6 cells/ml. Glial cell proliferation was prevented by
adding cytosine-arabino-furanoside-monophosphate (Sigma, St. Louis, MO), 19 hrs
after plating at a final concentration of 10 μ M. Cultures generated by this method
have been characterized and shown to contain more than 90% granule cells (2).

25 *Cell Toxicity Models*

Experiments were started after cells were 8 days in vitro (8 DIV). Cells were
washed with two ml of serum free growth medium. Concentrations of 100X stock
solutions of PPX and the (+) enantiomer in serum free growth medium were made.
These were delivered to cells by adding 20 μ l to cells in growth medium per well.
30 The final concentration of drugs ranged from 1 nM to 10 μ M. After 5 min 20 μ l of a
10X stock solution of L-dopa in serum free growth medium was added to each well
so that the final concentration was 100 μ M. Cells were incubated under the above
conditions for 24 hrs. For viability measurements cells were then washed twice and
pulsed with 1 μ Ci/ml of α -(methyl)- 14 C)-aminoisobutyric acid (New England
35 Nuclear) in Locke's buffer for 1 hr. Cells were solubilized, following a wash, with

0.5% Triton X-100. The solubilized cells were then counted for radioactivity on a scintillation counter. Data was expressed as the mean of triplicates \pm S.D. for each point.

Results from the table show that pramipexole was neuroprotective toward the toxicity (64.8 %) associated with L-dopa. At 10 μ M pramipexole, L-dopa treated cells were not different from controls. Pramipexole is shown to decrease slightly but significantly cAMP levels in cerebellar granular cells (3). This suggests the possible involvement of dopamine receptors (D_2 family) in the mechanism of neuroprotection. To test this hypothesis, the (+) enantiomer was tested in a parallel experiment with pramipexole. The (+) enantiomer has been shown to be inactive in a battery of binding assays that involve adrenergic and serotonergic receptors, and less active in dopaminergic receptors. The results show that the (+) enantiomer is equally as potent and effective compared to pramipexole as a neuroprotective agent in this assay.

The neuroprotective effects of pramipexole in L-dopa mediated toxicity in cerebellar granule cell does not appear to involve the activation of dopamine receptors.

The (+) enantiomer of pramipexole shows utility as a neuroprotectant despite the fact that it shows little ability to bind to monoamine receptors.

The possibility exists that pramipexole and its (+) enantiomer can act as an antioxidant toward L-dopa toxicity which is known to involve the generation of reactive oxygen species.

TABLE 1
Toxicity (64.8%) of 100 μ M L-dopa:
Effects of pramipexole and (+) enantiomer.

	Dose (nM)	Pramipexole (% of control*)	(+) enantiomer (% of control*)
30	0	35.2 \pm 8.5	35.2 \pm 8.5
	1	30.0 \pm 6.5	51.3 \pm 5.8
	10	39.2 \pm 9.4	57.8 \pm 4.9
	100	64.8 \pm 4.7	58.3 \pm 10.3
	300	76.2 \pm 7.3	81.0 \pm 18.3
35	1000	65.5 \pm 14.8	86.9 \pm 7.0
	3000	84.9 \pm 0.6	95.2 \pm 2.5
	10000	90.0 \pm 11.5	99.8 \pm 8.9

* Control is defined as the radioactivity associated with cells that were exposed to vehicle buffer rather than 100 μ M L-dopa. Control value was equal to an average of 129547 CPMs for triplicate counts.

CLAIMS

1. A method for preventing neuronal damage or the progression of neuronal damage in a patient suffering from or susceptible to such neuronal damage
5 comprising the administration of an effective amount of the compound 2-amino-6-n-propylamino-4,5,6,7-tetrahydrobenzothiazole and the pharmacologically acceptable acid addition salts thereof.
2. A method of claim 1 where the condition is selected from Parkinson's disease,
10 primary neurodegenerative disease; Huntington's Chorea; stroke and other hypoxic or ischemic processes; neurotrauma; metabolically induced neurological damage; sequelae from cerebral seizures; hemorrhagic stroke; secondary neurodegenerative disease (metabolic or toxic); Alzheimer's disease, other memory disorders; or
vascular dementia, multi-infarct dementia, Lewy body dementia, or neurogenerative
15 dementia.
3. A method of claim 2, where the condition is Parkinson's disease.
4. A method of claim 3, where the total dose of the compound is about
20 0.1-6 mg/day.
5. A method of claims 1-4, wherein the compound is the form of the (+) enantiomer.
- 25 6. A method of claims 1-4, wherein the compound is the form of the (-) enantiomer.
7. A method of claims 1-6, wherein the compound is the dihydrochloride.
- 30 8. Use of a compound selected from 2-amino-6-n-propylamino-4,5,6,7-tetrahydrobenzothiazole and the pharmacologically acceptable acid addition salts thereof for preparing a medicament for preventing neuronal damage or the progression of neuronal damage.
- 35 9. A use of claim 8, wherein the neuronal damage results from Parkinson's

disease, primary neurodegenerative disease; Huntington's Chorea; stroke and other hypoxic or ischemic processes; neurotrauma; metabolically induced neurological damage; sequelae from cerebral seizures; hemorrhagic stroke; secondary neurodegenerative disease (metabolic or toxic); Alzheimer's disease, other memory disorders; or vascular dementia, multi-infarct dementia, Lewy body dementia, or neurodegenerative dementia.

10. A use of claim 8, wherein the neuronal damage results from Parkinson's disease.
- 10 11. A use of claims 8-10, wherein the dosage is 0.1-6 mg/day.
12. A use of claims 8-11, wherein the compound is the (+) enantiomer.
- 15 13. A use of claims 8-12, wherein the compound is the (-) enantiomer.
14. A use of claims 8-13, wherein the compound is the dihydrochloride.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 95/15613

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K31/425

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DE,A,39 37 271 (BOEHRINGER INGELHEIM) 16 May 1991 cited in the application see the whole document ---	1-3, 6-10,13, 14
X	EP,A,0 186 087 (KARL THOMAE GMBH) 2 July 1986 cited in the application see page 13 - page 19 ---	1-3, 5-10, 12-14
X	CURR. OPIN. NEUROL. NEUROSURG., vol. 6, no. 3, 1993 pages 339-343, XP 000568176 A. LIEBERMAN 'Treatment of Parkinson's disease.' see page 341 ---	1-3,8-10
	-/--	

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

A document member of the same patent family

Date of the actual completion of the international search

11 April 1996

Date of mailing of the international search report

26, 04. 96

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Klaver, T

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 95/15613

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	<p>NEUROSCI. LETT., vol. 196, no. 1-2, 1995 pages 97-100, XP 000568217 M. CAMACHO-OCHOA ET AL. 'Rare brain binding sites for pramipexole, a clinically useful D3-preferring dopamine agonist.' see the whole document -----</p>	<p>1-3, 7-10,14</p>

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 95/15613

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
DE-A-3937271	16-05-91	AT-T- 108668	15-08-94
		AU-B- 635358	18-03-93
		AU-B- 6650890	16-05-91
		CA-A- 2029524	10-05-91
		DE-D- 59006506	25-08-94
		EP-A- 0428038	22-05-91
		ES-T- 2058725	01-11-94
		IL-A- 96276	28-11-94
		JP-A- 3170425	24-07-91
		US-A- 5112842	12-05-92
EP-A-186087	02-07-86	DE-A- 3447075	03-07-86
		DE-A- 3508947	18-09-86
		AU-B- 583874	11-05-89
		AU-B- 5154485	17-07-86
		CA-A- 1263653	05-12-89
		DK-B- 168862	27-06-94
		IE-B- 58863	17-11-93
		JP-C- 1854941	07-07-94
		JP-A- 61155377	15-07-86
		US-A- 4843086	27-06-89
		US-A- 4886812	12-12-89
		US-A- 4731374	15-03-88